DTIC FILE COPY

AD-A198 625

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION U	1b RESTRICTIVE MARKINGS NA
2a SECURITY CLASSIFICATION AUTHORITY NA	3 DISTRIBUTION / AVAILABILITY OF REPORT Distribution Unlimited
2b DECLASSIFICATION / DOWNGRADING SCHEDULE NA	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Montana State University: No. 1	5 MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a NAME OF PERFORMING ORGANIZATION Department of Microbiology Montana State University 6b OFFICE SYMBOL (If applicable) NA	7a NAME OF MONITORING ORGANIZATION Office of Naval Research
6c ADDRESS (City, State, and ZIP Code) 109 Lewis Hall Montana State University Bozeman, MT 59717	7b ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000
8a NAME OF FUNDING / SPONSORING ORGANIZATION Office of Naval Research 8b OFFICE SYMBOL (If applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-88-0103
8c ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 61153N PROJECT NO: RR0416 TASK NO: 4414003 WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)
The Use of a Sensory Model to Facilitate the Study of the Biochemistry of Adhesion to Surfaces in Marine Fouling Diatoms

12 PERSONAL AUTHOR(S)
Cooksey, B., Cooksey, K.E.

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Progress	FROM 11/87 TO 5/88	1988, 5, 31	7

16 SUPPLEMENTARY NOTATION
NA

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Fouling, diatom, sensory transduction, surfaces, chemical signals,

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The goal of this project is to understand the means by which fouling diatoms sense surfaces and adhere to them in their formation of a biofilm layer. We have begun to define the type of chemical signal needed by the diatom in order that it secrete adhesive. It is possible that the crucial elements of this signal can be obtained from the marine conditioning layer. The work depends on methodology wherein chemical gradients are presented to motile diatoms and their response determined using video microscopy and manual image analysis. Future work will concentrate on defining the specificity of putative membrane receptors from the organisms.

DTIC ELECTE JUN 21 1988 S D E

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION (u)
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. M. Marron	22b TELEPHONE (Include Area Code) (202) 696-4760 22c OFFICE SYMBOL ONR

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

PROGRESS REPORT ON CONTRACT N00014-88-0103 R&T Code 441H003
May 31, 1988

Co-Principal Investigators: B. Cooksey and K.E. Cooksey

CONTRACTOR: Montana State University

CONTRACT TITLE: The Use of a Sensory Model to Facilitate the Study of the Biochemistry of Adhesion to Surfaces in Marine Fouling Diatoms

START DATE: November 1, 1987

Research Objective: To investigate the means by which fouling diatoms sense and adhere to surfaces

Rationale for the Research:

Diatoms are found commonly on all illuminated surfaces in the sea, especially antifoulant coatings. Diatoms are implicated in detoxification of antifoulant coatings for other organisms and in pitting corrosion of ferrous metals. Unlike bacteria, diatoms are not inherently sticky. In order to adhere to a surface, diatoms must produce and secrete an adhesive. The production of such a substance by cells not on a surface would be energetically wasteful since these processes consume metabolic energy. They are also known to be calcium dependent. Because of this, it is reasonable to believe that the process of diatom adhesion is under metabolic regulatory control which further implies that adhesion must be initiated by an extracellular signal. The impetus for our investigation concerns the possibility that the reception and transduction of this signal by diatoms can be disrupted, leading to an inhibition of the adhesive process. Molecules able to carry out this disruption could be investigated as model antifoulants, which may be active for organisms other than diatoms, since the biochemistry of such basic processes as cellular adhesion tends to vary in detail only, from species to species.

Research Approach:

In our study of this problem, we have made two assumptions. The first of these is to consider that the signal is likely to be chemical in nature. There is ample precedent for this in the marine environment. The second concerns the diatom cell per se.

89 6 21 003

iatoms move by the secretion of a trail substance or macromolecular polymer. Diatoms are therefore incapable of movement unless they are first adhered to a surface. Our investigation of diatom taxis in response to chemical signals is therefore analogous to a situation where cellular adhesion is measured as a function of an extracellular chemical signal. Using a microscope-slide diffusion chamber, video-microscopy, tape recording and manual image analysis, we have defined some of structural characteristics required for a molecule to induce directed movement and thus adhesion.

Results: Year 1 (partial year)

Figure 1 summarizes the results of our experiments using sugars and their analogues. The dotted line around the 2 position of the pyranose ring indicates that one hydroxyl group is an alternate to the other. This then takes into account that mannose induces negative chemotaxis. The R- at the 4 position accommodates the fact that cells are also attracted to D-maltose as well as D-glucose and 3-0-methyl D-glucose. 2-Deoxy D-glucose was without effect. The -CHOH at position 5 shows the positive chemotaxis seen in D-glucoheptose gradients. We suggest that in view of the amelioration of the toxic effects of mannose with prior exposure to glucose and the fact that mannose causes negative taxis, there are at least two types of sugar receptors. One of these may be a general receptor for hexoses and the other for mannose and its analogues. It is difficult to see how a negatively chemotactic effect could be promoted by mannose without a unique receptor. Glucose would however, compete at the mannose receptor site.

From our knowledge concerning diatom adhesion, motility and chemosensing, we can suggest a conceptual model for the cascade of events in diatom adhesion to substratum that is consistent with that currently accepted for agonist-stimulated secretion coupling. In this theory, the binding of an agonist to a membrane receptor causes sustained entry of Ca^{2+} into cells from the extracellular space. This is achieved by a process whereby inositol triphosphate liberated enzymically from the plasma membrane acts as an intermediate transducer of the extracellular signal and causes the release of free Ca^{2+} from the internal non-mitochondrial Ca^{2+} pool. The emptying of this pool in turn causes it to be refilled from extracellular Ca^{2+} via a Ca-gate normally kept in the closed state by a filled internal pool, i.e., as long as an agonist is bound to the membrane, a continued transmembrane Ca^{2+}-flux is available to expedite intracellular Ca-dependent reactions. If such a system was present in diatoms and was involved in chemosensing, then binding of an agonist to the cell membrane or sensing of a surface-associated signal should also promote release of internal $Ca2^{+}$ in these organisms. This should happen whether or not external Ca^{2+} is sufficient to

SPECIFICITY OF PUTATIVE
CHEMOSENSORY RECEPTORS

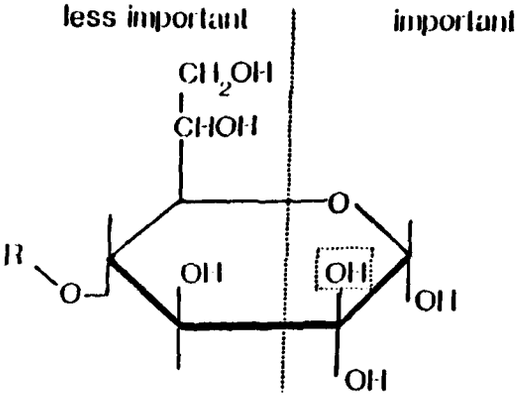


Fig. 1. Specificity of chemoreceptors for Amphora coffeaeformis.

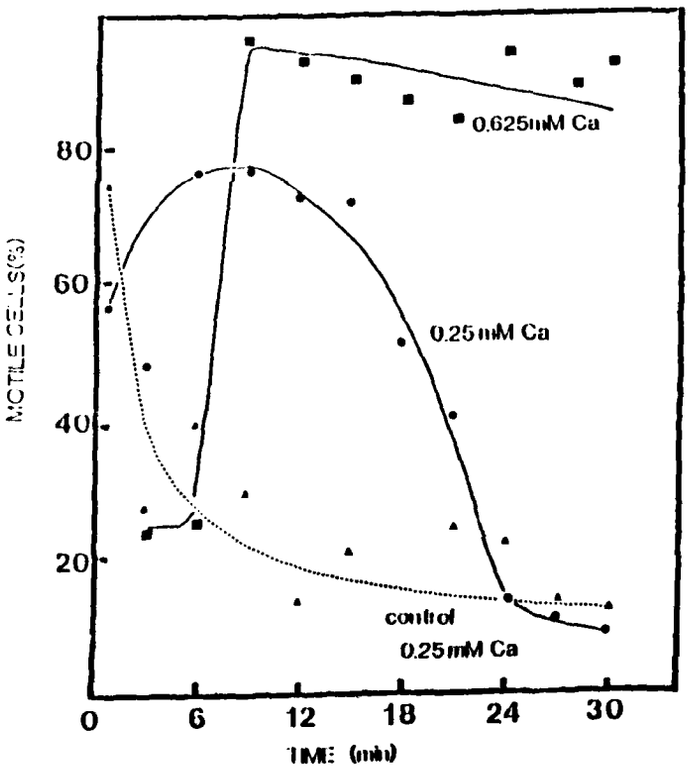


Fig. 2. Agonist-sustained motility with A. coffeaeformis. ▲, control cell in minimal medium without glucose; ●, cells in minimal medium with 1mM D-glucose; ■, minimal medium with 0.625mM Ca and 1mM D-glucose.

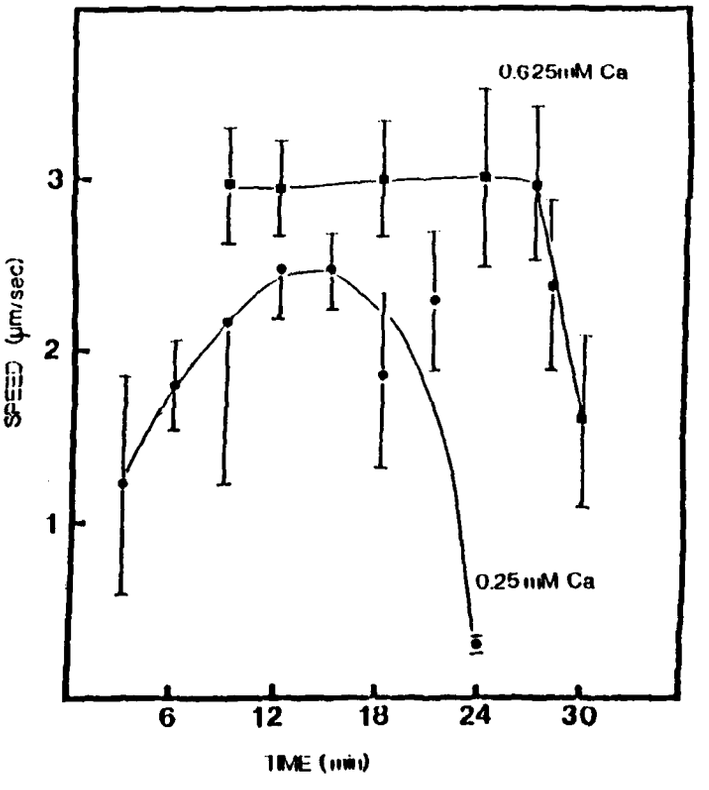


Fig. 3. Data obtained from experiment described in Fig. 2. Speed of motility measured on individual cells. ■, cells in minimal media containing 0.625mM Ca and 1mM D-glucose; ●, cells in minimal medium containing 0.25mM Ca and 1mM D-glucose.

refill the internal pool. If external Ca^{2+} is below a critical level to allow refilling of the pools, then Ca^{2+}-regulated processes may be activated, but the activation should be short-lived. Figures 1 and 2 suggest that this happens for glucose-sustained motility in Amphora coffeaeformis. The percentage of motile cells is increased by the presence of D-glucose, or most importantly for our future receptor work, by 3-O-methyl D-glucose, an unmetabolized analogue of glucose. The effect of glucose is enhanced at a slightly higher calcium concentration (0.625 mM). The speed of the cells in the presence of sugars was also increased for a short time before falling precipitously after about 20 min. There were no differences found between media containing 0.25 mM and 0.625 mM calcium when speed or percentage motility were measured in the absence of a sugar. This shows that the above results can be attributed to the presence of a sensed, but not necessarily metabolized, sugar. It was not possible to measure speed accurately in the absence of sugars because cells, although moving, did not move far enough before stopping for an accurate measurement to be obtained.

These results are discussed at length in the attached manuscript already submitted to Journal of Cell Science.

Research to be Carried Out in Balance of year 1 and in Year 2

The effects of the pretreatment with one sugar on chemosensing of another by cells of A. coffeaeformis is being investigated currently. This will provide information concerning specificity of receptors and the degree to which sugars can compete for occupancy of potential chemosensory receptors. Further, more direct competition experiments are planned as well as chemotactic studies with glucose oligomers. The work with oligomers relates to our hypothesis that the environmental surface signal is not a free-sugar, but a component of the marine conditioning layer. Pertinent experiments will be repeated with a further organism to provide some assurance of the general nature of our findings. We will also determine affinities of the putative receptors for at least two sugars (D-glucose and its non-metabolized analogue 3-O-methyl D-glucose). We expect this work to be performed much faster and more conveniently if we are successful in the DURIP competition where we have asked for image analysis equipment. This equipment will allow much more sophisticated questions to be asked concerning cellular reactions to signals than is possible at the moment. These are detailed in the proposal to DOD (information copy sent to Biosurfaces Program Managers).

We have begun our work concerning membrane preparations from A. coffeaeformis. We have scaled up our cell growth system so that an undergraduate student can now produce enough cells in one week for a membrane preparation experiment. In the coming months

and in Year 2, much of our time will be spent in developing methods for preparing membranes and determining their purity (i.e. freedom from other membrane fractions).

It appears that a French press will give the desired cell breakage. From this point, we intend to use centrifugal density gradients or an aqueous two-phase method for fractionation. In this latter method, the crude membrane fraction (microsomal fraction) is separated by surface change rather than density. The 2-phase system we will use initially will contain polyethylene glycol and dextran T500. We will follow the distribution of 5^{1}-nucleotidase and/or ion-mediated ATP-ases as markers of plasma membranes. In Year 2 we expect to be in a position to start membrane-agonist binding studies. The characteristics of the putative receptors (K_D, specificity) will be compared to similar parameters obtained in whole-cell chemosensory experiments and in experiments where transmembrane transport are measured. This will allow chemosensing receptors to be distinguished from membrane transport proteins. Since transmembrane transport of glucose is an inducible property in Amphora, and chemosensing appears not to be, it should be possible to distinguish between these phenomena at the membrane level.

Inventions

A patent has been applied for in conjunction with Drs. S. Smith, B. McLeod, and A. Liboff concerning "A System for Controlling Cell Behavior Using an Applied Oscillating Magnetic Field." Although the work in the application was supported to only a small extent by ONR, all the analyses needed in documenting the system were developed and published under ONR sponsorship.

Publications and Reports (partial Year 1)

1. Signal Response Behavior Demonstrated by Chemosensing in Diatoms of the Genus Amphora. Barbara Cooksey and K.E. Cooksey. J. Cell Science (submitted).

2. "Biofilm Formation and Interactions." Invited presentation at the New Orleans American Geophysical Union/Limnology and Oceanography meeting, January, 1988. Symposium Topic: Aquatic Microenvironments.

3. "The Nature of Surfaces and Biofilms," in American Society of Microbiology Regional Symposium on "A Cross-disciplinary Look at Cellular Adhesion." Bozeman, Montana. September 11, 1988.

4. Two papers have been submitted to the 7th International Congress on marine Corrosion and Fouling. Valencia, Spain, November, 1988. We are waiting for confirmation of their acceptance. Abstracts have been accepted already.

5. A Sensory Model for the Adhesion of Fouling Diatoms to Surfaces. Annual Meeting of the Phycological Society of America, Monterey, California. July, 1988.

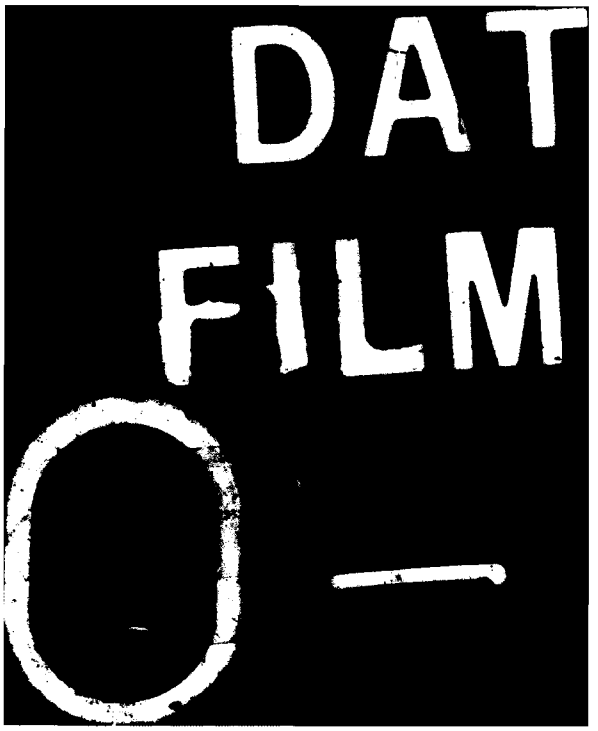
Training Activities

One undergraduate student has received training in algal microbiology and was supported by this program. Her contribution to the work was in growing large quantities of diatom cells for membrane isolation.

A second undergraduate student has undertaken a senior research project under the auspices of this program. He is investigating the effect of hexose on the initial attachment of diatoms to surfaces.

Accession For	
NTIS GRA&I	X
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

DTIC COPY INSPECTED 4


DAT
FILM